Effects of hydralazine on the elasticity of collagen[1]

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Summary. The elasticity module of collagen fibres from the tail tendon of the rat was reduced after incubation with hydralazine (10^{-4} to 10^{-1} mM/ml). It was also diminished by two metabolites of hydralazine.

After i.v. injection into rats, hydralazine[4] accumulates in the media of large arteries and veins, which is rich in elastin and collagen[5,6]. The present studies were undertaken to determine whether the binding of hydralazine to collagen may produce a change in the elasticity or other mechanical characteristics of the protein. In a simplified experimental model, the effect of hydralazine on collagen fibres, prepared from the tail tendon of the rat, was investigated.

Materials and methods. Long-Evans rats of an average weight of 300 g (about 10 months old) were killed by ether anaesthesia, and immediately afterwards 50 collagen fibres were prepared from the tendon of the tail and stored in a 3.5% gelatine solution (Haemaccel) at 4 °C. For each fibre, a stress-strain curve was established by repeated extension[7,8]. The slope S of the linear part of such a curve is directly related to the elasticity module of the fibre according to the formula

$$E = \frac{S}{A}, \qquad (1)$$

where E refers to the elasticity module and A to the cross-section area of the fibre. S corresponds to $\Delta\sigma/\Delta l$, where $\Delta\sigma$ indicates the change in tension and Δl the change in the length of the fibre. Each fibre was measured before and after the incubation with hydralazine. The relative diminution of the elasticity module was calculated according to the equation

$$-\frac{\Delta E}{E} = \frac{S_0 - S_1}{S_0}, \qquad (2)$$

where S_0 is the slope before and S_1 the slope after incubation.

Since the elasticity module depends on the pH-value of the incubation medium, each fibre was kept in Haemaccel for 24 h, at a pH corresponding to that of the incubation medium. In this way, an influence of the pH-value on the elasticity module could be excluded[9].

Results and discussion. In view of the instability of hydralazine[10] in aqueous solutions, which is affected by the pH-value, the ionic strength, and the buffers, the stability of hydralazine was studied in Haemaccel at pH 7.2, 6.8, and 6.3.

The results obtained confirm those of earlier investigators[10] that decomposing of hydralazine corresponds to zero-order kinetics. Since hydralazine is more stable in an acid medium, a pH of 6.6 was chosen for the incubation with the solutions of low concentrations.

An effect of the pH-value on the elasticity module was already evident at pH 6.5. An increase in acidity of the medium caused a reduction in the elasticity module. Such a pH-effect was only observed in Haemaccel, whereas in Ringer's solution, also at pH 5.5, no effect on the elasticity of collagen was demonstrable.

Hydralazine caused a dose-dependent decrease in the elasticity module (figure 1). Since the buffer capacity of Haemaccel is low, the pH-value of concentrated solutions of hydralazine was below 6.5. In figure 1, the lower curve corresponds to the sum of effects caused by hydralazine in an acid medium of pH 6.5. To exclude the pH-effect, all experiments were repeated at a constant pH of 7.0 (upper curve of figure 1).

The effect of hydralazine as a function of the hydrogen-ion concentration of the medium was also studied, and a linear relationship between the degree of the hydralazine effect

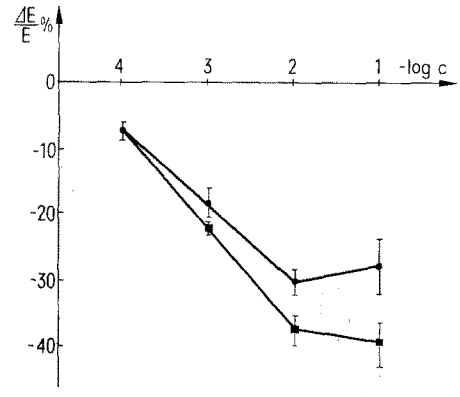

Fig. 1. Effect of hydralazine on the elasticity module of collagen fibres after incubation of 24 h at 25 °C in Haemaccel®. ● Upper curve: pH-value of the medium, not adjusted. ■ Lower curve: pH-value of the medium, adjusted to 6.6–7.0. Ordinate: Elasticity module in percent; abscissa: negative logarithm of hydralazine concentration (mM/l). Each point represents the mean value (±SE) of at least 5 experiments.

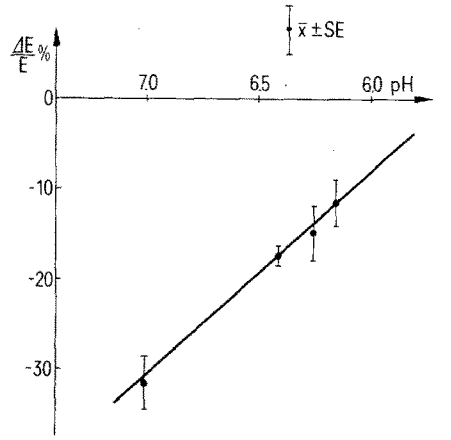

Fig. 2. Effect of hydralazine on the elasticity module as a function of the pH-value in the incubation medium. Incubation of collagen fibres for 24 h in Haemaccel®-solution containing 10^{-2} mM/ml hydralazine. Each point represents the mean value (±SE) of at least 4 experiments.

Effect of two metabolites of hydralazine on the elasticity module of collagen

Metabolite	mM/ml	$-\frac{\Delta E}{E}$ % ± SE
MTP	10^{-2}	21 ± 5
Acetonide	10^{-3}	25 ± 5

and the pH-value of the incubation medium was obtained (figure 2). It is presumed that, in an acid medium, the diffusion of the protonized hydralazine is more strongly inhibited than the diffusion of the free base present in a neutral medium.

Two metabolites of hydralazine, 3-methyl-s-triazolo-[3,4,a]-phthalazine (MTP) and hydralazine-acetonhydrazone (acetonide), were studied with respect to their effect on the elasticity module. None of the metabolites lowered the blood pressure in spontaneously hypertensive rats, but they caused a significant increase in the elasticity of collagen (table).

Whereas acetonide has a greater effect on the collagen than has a comparable concentration of hydralazine, the effect of MTP is definitely reduced.

The marked reduction in the elasticity module by metabolites which have no blood-pressure lowering effect, raises the question whether the effect of hydralazine on the mechanical features of collagen fibres is only of secondary significance for the overall action of hydralazine.

1 These investigations were supported by the Deutsche Forschungsgemeinschaft, No. 102/9.
2 Acknowledgment. The authors thank R. Jonack and Dr H. Riedl for technical assistance and helpful discussions.
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Toxicity of Dithane M-45 on *Drosophila melanogaster*

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Summary. The toxicity of different concentrations of Dithane M-45 on *Drosophila melanogaster* was determined. The chemical was administered by larval feeding. It has been estimated that the LC_{50} is 17.5 mg/100 ml food medium. The studies have suggested that Dithane M-45 has a pronounced effect on the rate of development and viability.

Pesticides are being used extensively in the control of crop pests and vector-borne diseases. Adequate mutagenicity testing on pesticides might be useful for the evaluation of their toxicities. Dithane M-45 is a new broadspectrum dithiocarbamate fungicide and a residual pollutant, which requires a continuous monitoring in the ecosystem. It has been shown that Dithane M-45 is toxic for the alga *Stichococcus bacillaris*[2] and least phytotoxic[3]. Studies of Vasudev and Krishnamurthy[4] have revealed that Dithane M-45 has an effect on crossing over in *D. melanogaster*. The knowledge on the toxicological effects of this environmental pollutant on animals and man is very meagre. The authors therefore considered it interesting to report here the findings on the action of Dithane M-45 on the rate of development and viability in *D. melanogaster*.

Materials and methods. *D. melanogaster*, Oregon-K strain, was used in the present investigations. The eggs were collected following the procedure of Delcour[5]. Eggs of the same age (±4 h) and in equal numbers (35 eggs/vial) were placed in normal and Dithane M-45 (Indofil Chemicals Limited, Bombay) supplemented media. Different concentrations of 2, 5, 10, 15, 17.5, 20, 25 and 30 mg of the chemical were thoroughly mixed in 100 ml wheat cream agar medium. 700 eggs were alloted to each group. The normal medium was used as control.

Flies were counted every day from the first day of eclosion to the last day of emergence. Sexes were noted. From this data, mean developmental time of the whole group, as well of sexes, survival value and sex-ratio were calculated. All the experiments were carried out at a constant temperature of 23±1 °C.

Results and discussion. As the rate of development is one of the parameters by which the toxicity of the chemical is measured, the effect of various concentrations of Dithane M-45 on rate of development has been analyzed. The table incorporates the data on the mean developmental time in

Toxicity of Dithane M-45 on *D. melanogaster*

Concentration in food agar	Mean developmental time for group	Number of adults emerged out of 700 eggs laid		Mean number of offspring per vial	Percentage lethality
		M	F		
Control	11.51±0.07	331	324	32.75±0.45	6.43
2 mg/100 ml (20 ppm)	15.29±0.17*	292	283	28.75±1.32	17.86
5 mg/100 ml (50 ppm)	15.98±0.07*	271	257	26.40±1.24	24.58
10 mg/100 ml (100 ppm)	16.49±0.08*	246	229	23.75±2.05***	32.15
15 mg/100 ml (150 ppm)	17.72±0.12*	203	184	19.35±1.05***	44.72
20 mg/100 ml (200 ppm)	18.59±0.23*	168**	130**	14.90±2.86***	57.43
25 mg/100 ml (250 ppm)	21.72±0.24*	68	71	6.95±2.22***	80.15
30 mg/100 ml (300 ppm)	24.44±0.24*	51	44	4.75±1.35***	86.43

* Control versus treatment significant at 5% level. ** Sex-ratio is significant; $p < 0.05$. *** Control versus treatment; by analysis of variance: $p < 0.05$. F = Female, M = male.